L2: Entry 3 of 3 File: USPT Mar 18, 2003

US-PAT-NO: 6534300
DOCUMENT-IDENTIFIER: US 6534300 B1

TITLE: Methods for producing highly phosphorylated lysosomal hydrolases

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/195; 435/194

CLAIMS:

I claim:

1. A method of modifying a lysosomal hydrolase comprising contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphotransferase to produce a modified lysosomal hydrolase, wherein said N-acetylglucosamine-phosphotransferase comprises SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

2. The method of claim 1, further comprising purifying said modified lysosomal hydrolase after said contacting.

3. The method of claim 1, wherein said lysosomal hydrolase comprises an asparagine-linked oligosaccharide with a high mannose structure.

4. The method of claim 1, wherein said N-acetylglucosamine-phosphotransferase catalyzes the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the hydrolase.

5. The method of claim 1, wherein said lysosomal hydrolase is a recombinant hydrolase.

6. The method of claim 1, wherein said N-acetylglucosamine-phosphotransferase comprises amino acids 1-928 of SEQ ID NO:1, amino acids 1-328 of SEQ ID NO:2, and amino acids 25-305 of SEQ ID NO:3.

7. The method of claim 1, wherein said lysosomal hydrolase is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetlygalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B,

Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase G.sub.M1 Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase .beta.-Glucosidase.

8. The method of claim 7, wherein said lysosomal hydrolase is .alpha.-glucosidase.

9. A phosphorylated purified lysosomal hydrolase comprising a mannose 6-phosphate, which comprises at least 6% bis-phosphorylated oligosaccharides.

10. The phosphorylated purified lysosomal hydrolase of claim 9, which comprises up to 100% bis-phosphorylated oligosaccharides.

11. The phosphorylated purified lysosomal hydrolase of claim 9, which comprises at least 5 mannose 6-phosphates.

12. The phosphorylated purified lysosomal hydrolase of claim 9, which is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetlygalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase G.sub.M1 Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase .beta.-Glucosidase.

13. The phosphorylated purified lysosomal hydrolase of claim 12, which is .alpha.-glucosidase.

14. A method of preparing a phosphorylated lysosomal hydrolase comprising contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase, wherein said lysosomal hydrolase comprises a terminal mannose 6-phosphate, and said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase comprises the amino acid sequence in SEQ ID NO:6.

15. The method of claim 14, wherein said method further comprises purifying the phosphorylated lysosomal hydrolase.

16. The method of claim 14, wherein said lysosomal hydrolase is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetlygalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase G.sub.M1 Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and

Glucocerebrosidase .beta.-Glucosidase.

17. The method of claim 14, wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

18. The method of claim 14, wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase comprises amino acids 50-515 of SEQ ID NO:6.

19. A lysosomal hydrolase obtained by the method of claim 14.

20. The method of claim 16, wherein said lysosomal hydrolase is .alpha.-glucosidase.

21. A method of preparing a phosphorylated lysosomal hydrolase comprising: contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-phosphotransferase to produce a modified lysosomal hydrolase, wherein said N-acetylglucosamine-phosphotransferase comprises SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3; and contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase, wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase comprises the amino acid in SEQ ID NO:6.

22. The method of claim 21, further comprising purifying said phosphorylated lysosomal hydrolase after said contacting with the isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase.

23. The method of claim 21, further comprising purifying said modified lysosomal hydrolase prior to said contacting with the isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase.

24. A phosphorylated lysosomal hydrolase obtained by the method of claim 21.

25. The method of claim 21, wherein said N-acetylglucosamine-phosphotransferase comprises amino acid 1-928 of SEQ ID NO:1, amino acids 1-328 of SEQ ID NO:2, and amino acids 25-305 of SEQ ID NO:3.

26. The method of claim 21, wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase comprises amino acids 50-515 of SEQ ID NO:6.

27. The method of claim 21, wherein said lysosomal hydrolase is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetlygalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase G.sub.M1 Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase .beta.-Glucosidase.

28. The method of claim 27, wherein said lysosomal hydrolase is .alpha.-glucosidase.

29. A method of modifying a lysosomal hydrolase comprising contacting said lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphotransferase to produce a modified lysosomal hydrolase, wherein said N-acetylglucosamine-phosphotransferase comprises an amino acid sequence that is at least 70% identical to SEQ ID NO:1, an amino acid sequence that is at least 70% identical to SEQ ID NO:2, and an amino acid sequence that is at least 70% identical to SEQ ID NO:3, and said N-acetylglucosamine-phosphotransferase catalyzes the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the lysosomal hydrolase.

30. The method of claim 29, further comprising purifying said modified lysosomal hydrolase after said contacting.

31. The method of claim 29, wherein said lysosomal hydrolase comprises an asparagine-linked oligosaccharide with a high mannose structure.

32. The method of claim 29, wherein said lysosomal hydrolase is a recombinant hydrolase.

33. The method of claim 29, wherein said lysosomal hydrolase is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetlygalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase GM, Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase .beta.-Glucosidase.

34. The method of claim 33, wherein said lysosomal hydrolase is .alpha.-glucosidase.

35. The method of claim 29, further comprising contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase, which comprises an amino acid sequence that is at least 70% identical to SEQ ID NO:6, and wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

36. The method of claim 35, further comprising purifying said lysosomal hydrolase after contacting with the isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase.

Previous Doc Next Doc Go to Doc#

L2: Entry 2 of 3 File: USPT Dec 30, 2003

US-PAT-NO: 6670165
DOCUMENT-IDENTIFIER: US 6670165 B2

TITLE: Methods for producing highly phosphorylated lysosomal hydrolases

DATE-ISSUED: December 30, 2003

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/195

CLAIMS:

What is claimed is:

1. A method of producing a modified lysosomal hydrolase comprising culturing transformed cells comprising a recombinant polynucleotide encoding a lysosomal hydrolase in the presence of an .alpha.1,2-mannosidase inhibitor; recovering the lysosomal hydrolase from the cultured transformed cells; and contacting the recovered lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphotransferase to produce a modified lysosomal hydrolase, wherein said N-acetylglucosamine-phosphotransferase comprises SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

2. The method of claim 1, further comprising purifying said modified lysosomal hydrolase after said contacting.

3. The method of claim 1, wherein said N-acetylglucosamine-phosphotransferase catalyzes the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the lysosomal hydrolase.

4. The method of claim 1, wherein said N-acetylglucosamine-phosphotransferase comprises amino acids 1-928 of SEQ ID NO:1, amino acids 1-328 of SEQ ID NO:2, and amino acids 25-305 of SEQ ID NO:3.

5. The method of claim 1, wherein said lysosomal hydrolase is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase G.sub.MI Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylglucosamine amidase, Acid Lipase, Acid

Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase .beta.-Glucosidase.

6. The method of claim 1, further comprising contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase, which comprises the amino acid sequence in SEQ ID NO:6.

7. The method of claim 6, wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolase and generates a terminal mannose 6-phosphate on said hydrolase.

8. The method of claim 6, wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase comprises amino acids 50-515 of SEQ ID NO:6.

9. The method of claim 1, wherein said alpha 1,2-mannosidase inhibitor is selected from the group consisting of deoxymannojirimycin, kifunensine, D-Mannonolactam amidrazone and N-butyl-dexoymannojirimycin.

10. The method of claim 1, wherein the alpha 1,2-mannosidase inhibitor is kifunensine.

11. The method of claim 1, wherein the alpha 1,2-mannosidase inhibitor is deoxymannojirimycin.

12. The method of claim 6, wherein said lysosomal hydrolase is .alpha.-glucosidase.

13. A modified lysosomal hydrolase obtained by the method of claim 1.

14. A modified lysosomal hydrolase obtained by the method of claim 2.

15. A modified lysosomal hydrolase obtained by the method of claim 3.

16. A modified lysosomal hydrolase obtained by the method of claim 4.

17. A modified lysosomal hydrolase obtained by the method of claim 5.

18. A modified lysosomal hydrolase obtained by the method of claim 6.

19. A modified lysosomal hydrolase obtained by the method of claim 7.

20. A modified lysosomal hydrolase obtained by the method of claim 8.

21. A modified lysosomal hydrolase obtained by the method of claim 9.

22. A modified lysosomal hydrolase obtained by the method of claim 10.

23. A modified lysosomal hydrolase obtained by the method of claim 11.

24. A modified lysosomal hydrolase obtained by the method of claim 12.

25. A method of modifying a lysosomal hydrolase comprising culturing

transformed cells comprising a recombinant polynucleotide encoding a lysosomal hydrolase in the presence of an .alpha.1,2-mannosidase inhibitor; recovering the lysosomal hydrolase from the cultured transformed cells; and contacting said recovered lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphotransferase to produce a modified lysosomal hydrolase, wherein said N-acetylglucosamine-phosphotransferase comprises an amino acid sequence that is at least 70% identical to SEQ ID NO:1, an amino acid sequence that is at least 70% identical to SEQ ID NO:2, and an amino acid sequence that is at least 70% identical to SEQ ID NO:3, and said N-acetylglucosamine-phosphotransferase catalyzes the transfer of N-acetylglucosamine-1-phosphate from UDP-N-Acetylglucosamine to a mannose on the lysosomal hydrolase.

26. The method of claim 25, further comprising purifying said modified lysosomal hydrolase after said contacting.

27. The method of claim 25, wherein said lysosomal hydrolase is selected from the group consisting of .alpha.-glucosidase, .alpha.-iduronidase, .alpha.-galactosidase A, arylsulfatase, N-acetylgalactosamine-6-sulfatase, .beta.-galactosidase, iduronate 2-sulfatase, ceramidase, galactocerebrosidase, B-glucoronidase, Heparan N-sulfatase, N-Acetyl-.alpha.-glucosaminidase, Acetyl CoA-.alpha.-glucosaminide N-acetyl transferase, N-acetyl-glucosamine-6 sulfatase, Galactose 6-sulfatase, Arylsulfatase A, Arylsulfatase B, Arylsulfatase C, Arylsulfatase A Cerebroside, Ganglioside, Acid .beta.-galactosidase G.sub.MI Galglioside, Acid .beta.-galactosidase, Hexosaminidase A, Hexosaminidase B, .alpha.-fucosidase, .alpha.-N-Acetyl galactosaminidase, Glycoprotein Neuraminidase, Aspartylgiucosamine amidase, Acid Lipase, Acid Ceramidase, Lysosomal Sphingomyelinase, Sphingomyelinase, and Glucocerebrosidase .beta.-Glucosidase.

28. The method of claim 25, wherein said lysosomal hydrolase is .alpha.-glucosidase.

29. The method of claim 25, further comprising contacting said modified lysosomal hydrolase with an isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase which comprises an amino acid sequence that is at least 70% identical to SEQ ID NO:6, and wherein said N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase catalyzes the removal of N-acetylglucosamine from said modified lysosomal hydrolases and generates a terminal mannose 6-phosphate on said hydrolase.

30. The method of claim 29, further comprising purifying said lysosomal hydrolase after contacting with the isolated N-acetylglucosamine-1-phosphodiester .alpha.-N-Acetylglucosamindase.

31. The method of claim 25, wherein said alpha 1,2-mannosidase inhibitor is selected from the group consisting of deoxymannojirimycin, kifunensine, D-Mannonolactam amidrazone and N-butyl-dexoymannojirimycin.

32. The method of claim 25, wherein the alpha 1,2-mannosidase inhibitor is kifunensine.

33. The method of claim 25, wherein the alpha 1,2-mannosidase inhibitor is deoxymannojirimycin.

34. A modified lysosomal hydrolase obtained by the method of claim 25.

35. A modified lysosomal hydrolase obtained by the method of claim 26.

36. A modified lysosomal hydrolase obtained by the method of claim 27.

37. A modified lysosomal hydrolase obtained by the method of claim 28.

38. A modified lysosomal hydrolase obtained by the method of claim 29.

39. A modified lysosomal hydrolase obtained by the method of claim 30.

40. A modified lysosomal hydrolase obtained by the method of claim 31.

41. A modified lysosomal hydrolase obtained by the method of claim 32.

42. A modified lysosomal hydrolase obtained by the method of claim 33.

Previous Doc Next Doc Go to Doc#